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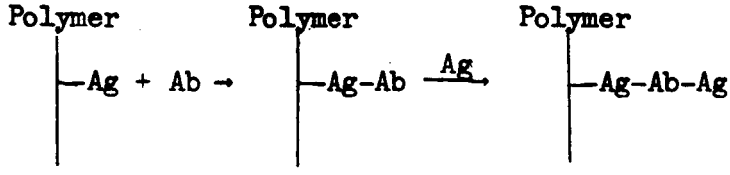
THE COUPLING OF BIOLOGICALLY ACTIVE SUBSTANCES TO INSOLUBLE POLYMERS: ANTIBODY ON CELLULOSE

In a search for improved methods of isolating pure antibody from serum, we have been investigating techniques for the chemical coupling of proteins and organic molecules to cellulose, carboxymethylcellulose, fibroin (silk)[1], and wool[2]. We have found that proteins and organic molecules with basic primary amino groups can be chemically coupled to carboxymethylcellulose in the presence of N,N'-dicyclohexylcarbodiimide, and that nonspecific adsorption of serum proteins to columns of such substances is negligible[1]. It became of interest to determine whether biologically active molecules could be coupled to and recovered from insoluble substances without loss of activity. For this purpose we chose to use rabbit antibody for the isolation of specific antigen.

Previous workers have used a "sandwich" method[3,4,5]. This technique involves chemically linking or adsorbing the antigen to the polymer and using this immunoadsorbent to complex antibody. Because of the bivalency of antibody, one active site remains available and is then used to complex added antigen. Schematically the method is as follows:



However the added antigen which complexed to the antibody was not recovered in any of the experiments[3,4,5]. One recent attempt to use immune globulins chemically linked to cellulose resulted in complexing of the antigen, however again the antigen could not be recovered[6]. A very recent report describes the recovery of antigen from complexes formed with an insoluble

polymer made by cross linking immune serum globulins with tetrazotized benzidine[7].

We have been able to demonstrate that antibodies can be coupled to cellulose with retention of activity and that complexed antigen can be recovered. We expect such substances to be useful for the study of the reactions of antigens with antibodies and for the isolation or purification of organic molecules and biological (or other) polymers to which antibody can be produced.

One gram of carboxymethylcellulose (0.78 meq/g) was coupled to benzidine by forming the monobenzidine amide derivative in the presence of N,N'-dicyclohexylcarbodiimide as previously described[8]. The free amino groups on the aminoarylcellulose were diazotized and the resulting material added to gamma globulin precipitated from the pooled sera of rabbits immunized against human gamma globulin. Those diazonium groups which did not couple to protein were blocked by reaction with β-naphthol. Approximately one-third of the gamma globulin fraction used was anti-human gamma globulin antibody.

A column was prepared using 0.5 g of the cellulose coupled to the immune globulins. After washing with 1% NaCl solution, 5 ml of a 0.2% solution of human gamma globulin was passed through the column, followed by 1% NaCl until no protein in the effluent was detectable spectrophotometrically at 220 mμ in a 1 mm continuous flow cell. The column was then eluted with 1% NaCl adjusted to pH 2.3 with HCl. Eluted protein, determined by the Lowry method[9], totaled 0.4 mg. Using sheep anti-rabbit gamma globulin serum, no rabbit gamma globulin could be detected in the eluate.

Using the same column, the experiment was repeated with bovine serum albumin substituted for human gamma globulin. Bovine serum albumin would not be expected to complex specifically with anti-human gamma globulin

antibody under these conditions. No protein could be detected in the pH 2.3 effluent either spectrophotometrically or by the Lowry method.

To eliminate any doubt that the protein eluted at pH 2.3 had been complexed with bound antibody, the same quantity of human gamma globulin solution was again passed through the column. This time 0.12 mg of protein was eluted at pH 2.3. The decreased activity of the bound antibody could be due to many causes, including differences in the rate of passage of antigen through the column, partial denaturation in acid solution or irreversible binding to antigen[10].

Antibodies coupled to insoluble carriers such as cellulose may be useful for the isolation or purification of substances with antigenic properties which are difficult to handle by other methods, after optimum conditions for stability and yield have been determined. Insoluble antibody derivatives, such as the one described, may be used for antigen-antibody dissociation studies or to investigate antibody denaturation mechanisms by agents which cause loss of activity. The coupling of individual antibody molecules to cellulose removes ambiguities arising from the possible unavailability to reaction of antibody molecules buried in the middle of antigen-antibody precipitates or of antibody polymers.

We are in the process of investigating the coupling of enzymes and other biologically active molecules to insoluble carriers and studying the behavior, activity and stability of the coupled species.

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